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【 ORIGINAL ARTICLE 】

The Frequency and Amount of Fish Intake Are Correlated with the White Blood Cell Count and Aerobic Exercise Habit: A Cross-sectional Study

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Abstract:

Objective We investigated the relationship between the amount and frequency of fish intake, and the white blood cell (WBC) count and aerobic exercise habits.

Methods We conducted a cross-sectional study between April 2019 and March 2020 at the Health Planning Center of Nihon University Hospital on a cohort of 8,981 male subjects.

Results The average amount and frequency of fish intake were 134±85 g/week and 2.14±1.28 days/week, respectively. The WBC count decreased significantly as the amount of fish intake increased (p<0.0001). According to a multivariate regression analysis, a high fish intake amount (β=-0.082, p<0.0001) and regular aerobic exercise (β=-0.083, p<0.0001) were independent determinants of a low WBC count. The proportion of subjects engaged in regular aerobic exercise increased with an increase in the amount of fish intake (p<0.0001). Furthermore, the amount and frequency of fish intake significantly correlated with the amount of n-3 polyunsaturated fatty acid intake determined using the Japan's National Nutrition Survey results (both r=0.962 and 0.958). Therefore, the amount of fish intake could be substituted by the average number of days of fish intake per week.

Conclusion A high fish intake was an independent determinant of a low WBC count and engagement in regular aerobic exercise, regardless of whether the fish intake was defined by the amount or frequency of fish intake. However, since fish intake frequency can be measured more easily, this may be used to measure the fish intake.

Key words: aerobic exercise habit, atherosclerotic cardiovascular disease, fish intake, white blood cell count

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Introduction

Chronic low-grade inflammation is associated with atherosclerosis. Inflammatory cells such as white blood cells (WBC) play an essential role in the development of atherosclerosis in the arterial vessel wall (1). Thus, an elevated WBC count is associated with an increased risk for cardiovascular diseases (2).

Epidemiological studies have demonstrated that an increased intake of fish rich in n-3 polyunsaturated fatty acid (n-3 PUFA), which had anti-inflammatory activity, was associated with a decreased risk for atherosclerotic cardiovascular diseases (ASCVD) (3-5). In addition, numerous published studies have reported that consuming non-fried seafood, especially those rich in n-3 PUFA, once or twice a week conferred cardiovascular benefits (6).

We recently showed that frequent fish intake that measured by the average number of days of fish intake per week was an independent determinant of a reduced WBC count and an improvement in the serum lipid profile. Moreover, frequent fish intake was associated with regular aerobic ex-

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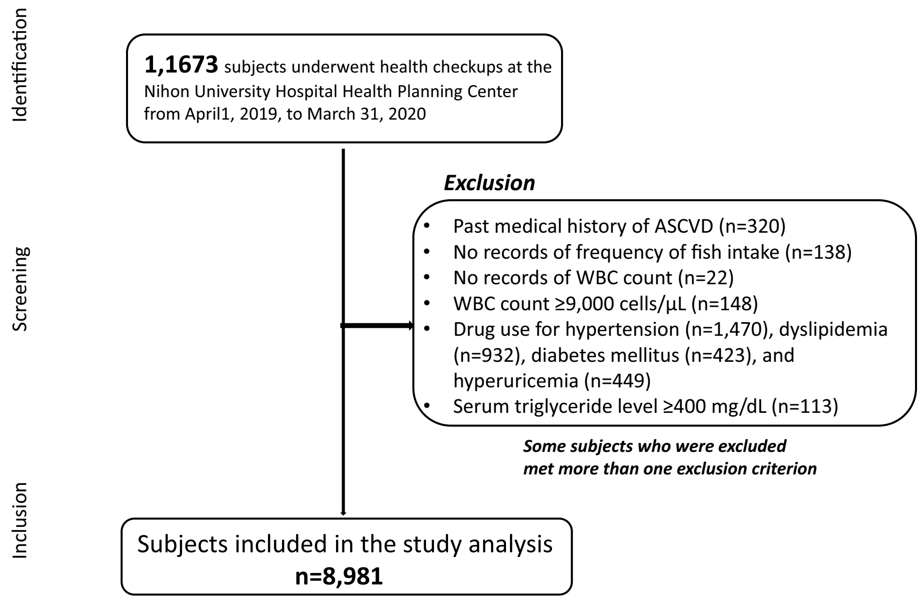

Figure 1. Flow diagram of the study participants. ASCVD: atherosclerotic cardiovascular disease, WBC: white blood cell

ercise habits, which indicates a healthy lifestyle (7-9). However, it was unclear whether the self-reported frequency of fish intake obtained from the questionnaire accurately reflected the amount of fish intake. Moreover, the frequency of fish intake as a general measure of fish intake may lead to some misinterpretations of the study results.

Large-scale cohort studies verified the protective effect of fish intake measured by the amount or frequency of fish intake on coronary artery disease development (CAD) (4). These studies showed that an increase in the amount and high frequency of fish intake reduced the risk for CAD. However, only a few studies have simultaneously examined the suppressive effect of the amount and frequency of fish intake as surrogate markers for ASCVD.

We hypothesized that an increase in the amount and frequency of fish intake is related to a reduction of WBC count and an increase in the likelihood of engaging in regular aerobic exercises. Moreover, the amount and frequency of fish intake as measures of overall fish intake result in similar findings. Therefore, the accuracy of fish intake frequency, which can be easily obtained from the study participants, can be considered an indicator of fish intake.

This study aimed to examine the relationship between the amount and frequency of fish intake, WBC count, and aerobic exercise habits and discuss the significance of using the two measurements for fish intake, the amount and the frequency.

Materials and Methods

Study design and study populations

In this cross-sectional study, we investigated the association of the average fish intake per week to measure the amount of fish intake: the average number of days of fish intake per week to measure the frequency of fish intake, and aerobic exercise on the WBC count in healthy Japanese subjects.

Among 11,673 Japanese subjects who underwent their annual health checkups between April 2019 and March 2020 at the Health Planning Center of Nihon University Hospital, we included 8,981 healthy males in this study. The exclusion criteria were as follows: unwilling to provide consent for participation in the study; current intake of lipid-modifying, antihypertensive, antidiabetic, and antihyperuricemic drugs; positive history of ASCVD; a serum triglyceride level of ≥400 mg/dL or a blood WBC count of ≥9,000 cells/μL, which is suggestive of infection; and no record on the frequency of fish intake. Fig. 1 shows the flow diagram for the selection of study participants.

The primary endpoint of the study was the relationship between fish intake (amount and frequency), daily aerobic exercise, and the WBC count. The secondary endpoint was the association between two indices of fish intake and daily aerobic exercise on WBC count. Furthermore, to determine the correlation between the amount and the frequency of fish intake, we examined the relationship between these two indices of fish intake based on the intake of n-3 PUFA obtained from the National Institute of Health and Nutrition based on the National Nutrition Survey results from the Ministry of Health and Welfare of Japan (10, 11).

This study was conducted in accordance with the ethical principles of the 1964 Declaration of Helsinki and its amendments. The Nihon University Hospital Ethics Committee approved the design and objectives. The study was retrospectively registered on February 1, 2020 with a Clinical Trial Registration of UMIN (http://www.umin.ac.jp/) (study ID: UMIN000039197).

Questionnaire to determine health behaviors

Trained interviewers conducted health behavior surveys at our institute via face-to-face interviews with the subjects. The surveys consisted of comprehensive questions designed to assess the subject's demographic and socioeconomic characteristics, including age, occupation, marital status, lifestyle behavior, medications, and medical and family history.

The subjects who underwent health checkups were given the lifestyle questionnaire and asked to provide appropriate responses. These questions are similar to the self-reported questionnaire described in our previous study (7-9).

1. Smoking habit: Do you smoke regularly? - No/ Yes/ I have quit smoking/ I quit smoking (_) years ago.

2. Drinking habit: Please indicate the frequency at which you drink - every day/ sometimes/ I used to drink, but have stopped drinking/ I stopped drinking (_) years ago/ I drink rarely/ I cannot drink; How much do you drink daily? [ethanol equivalent (g/day)]: <20 g/20 to <40 g/40 to <60 g/≥60 g: How many days per week do you drink?

3. Aerobic exercise habit: Do you exercise in a manner that makes you sweat slightly for 30 minutes a day at least twice a week for one year?

4. Fish intake: On average, how many days per week do you eat fish in the past month? Fish intake was assessed in weekly frequencies in the questionnaire (0, 1, 2, 3, 4, 5, 6, and approximately every day).

The questionnaire was a modified version of the Questionnaire on Specific Health Examination, which was used for specific health guidance during health checkups under the jurisdiction of the Ministry of Health, Labor and Welfare (MHLW) in Japan (12). The questionnaire mentioned above was an excerpt of questions relevant to this study from our institution's questionnaire (Supplementary material 1).

Assessment of estimated weekly average amount of fish intake

The National Nutrition Survey, led by the MHLW in Japan, has a standardized method of data collection and established quality control (13-15). Based on this survey record, the National Health and Nutrition Survey of Japan estimated the average daily amount of fish intake according to age group by estimating the "net food supply per person yearly of fish products for human consumption" based on domestic fish production, import and export information, changes in stocks, and population, among others. (Supplementary material 2) (10, 16). Then, we calculated the estimated weekly average amount of fish intake as follows:

The estimated weekly average amount of fish intake
= daily average amount of fish intake according to age
× weekly average number of days of fish intake

We categorized the weekly average amount of fish intake into seven variables: (1) <50 g/week; (2) ≥50 g/week but <100 g/week; (3) ≥100 g/week but <150 g/week; (4) ≥150 g/week but <200 g/week; (5) ≥200 g/week but <250 g/week; (6) ≥250 g/week but <300 g/week; and (7) ≥300 g/week.

Assessment of the estimated weekly average amount of estimated n-3 PUFA

We calculated the estimated weekly intake of n-3 PUFAs similar to the previously described method of calculating the amount of fish intake by age and sex per week. That is, based on the National Institute of Health and Nutrition, Section of the National Nutrition and Health Survey record (Supplementary material 3) (11), we calculated the estimated weekly average amount of n-3 PUFA as follows:

The estimated weekly average amount of n-3 PUFA
= daily average amount of n-3 PUFA according to age
× weekly average number of days of fish intake

Health examinations and blood samples

We measured the anthropometric variables of the subjects in a standing position using standardized techniques and equipment such as height and weight. Then, we calculated the body mass index by dividing the body weight, in kilograms, by the height, in meters, squared (kg/m^2). Next, using a standard mercury sphygmomanometer, blood pressure was measured twice - the first reading after a five-minute rest period and the second was after 3 minutes. We used the average of the first and second measurements in our analysis. Fasting blood samples were collected early in the morning after the subjects fasted for eight hours. At the central clinical laboratory of our institute, WBC count was determined using the Beckman Coulter STKS (Beckman Coulter Fullerton, USA).

Statistical analyses

Data were expressed as the mean ± standard deviation for continuous variables and as percentages for discrete variables.

A subset analysis to compare continuous variables according to the WBC count group was performed using one-way analysis of variance (ANOVA). We applied the Tukey-Kramer adjustment and Games-Howell adjustment as a post hoc analysis for covariates if there were differences in patient characteristics or laboratory profiles. We subsequently performed the chi-square test to compare categorical variables.

We performed a univariate linear regression analysis using WBC count as the dependent variable, and age, body mass index, serum lipid, blood pressure, heart rate, hemoglobin A 1c, fasting blood glucose level, uric acid level, and lifestyle behavior, which was measured as the average weekly amount of fish intake, the presence or absence of a cigarette smoking habit, and the aerobic exercise habit, as independent variables (model 1). Similarly, univariate and multivariate linear regression analyses were performed using the weekly average number of days of fish intake as dependent variables (model 2). Factors identified as significant were those with a $p<0.05$ in the univariate linear regression analysis. They were subsequently included in the multivariate linear regression analysis model. A two-way ANOVA was used

Table 1. Subject Characteristics and Laboratory Profiles according to the WBC Count as a Categorical Variable.

	WBC count						
	All cases (n=8,981)	Group 1 (n=1,810)	Group 2 (n=1,819)	Group 3 (n=1,741)	Group 4 (n=1,883)	Group 5 (n=1,728)	p value
	WBC count (range) (cells/μL)	1,700-3,800	3,900-4,400	4,500-5,000	5,100-5,900	6,000-8,900	
Male/female, n (%)	5,295 (59.0)/ 3,866 (41.0)	1,044 (57.7)/ 766 (4.3)	1,051 (57.8)/ 768 (42.2)	1,042 (59.9)/ 699 (40.1)	1,114 (59.2)/ 769 (40.8)	1,044 (60.4)/ 664 (39.6)	0.356
Age (years)	46.9±12.9	46.7±13.2	46.1±13.1	46.9±12.6	46.9±12.7	47.3±13.0	0.547
Age group, years, n (%)							0.434
20-<30	914 (10.2)	201 (11.1)	203 (11.2)	162 (9.3)	181 (9.7)	167 (10.2)	
30-<40	1,826 (20.3)	362 (20.0)	373 (20.5)	370 (20.3)	389 (21.3)	332 (18.2)	
40-<50	2,404 (26.8)	497 (20.7)	472 (19.6)	461 (19.2)	509 (21.2)	465 (19.3)	
50-<60	2,170 (24.4)	402 (18.5)	433 (20.0)	451 (20.8)	463 (21.3)	421 (19.4)	
60-<70	1,369 (15.2)	286 (15.8)	285 (20.8)	238 (17.4)	276 (20.2)	284 (20.7)	
≥70	298 (3.3)	62 (3.4)	53 (2.9)	59 (3.4)	65 (3.5)	59 (3.4)	
eGFR (mL/min/1.73 m²)	78.1±14.8	77.8±14.6	78.0±14.7	77.4±15.0	77.6±14.4	78.1±14.2	0.685
Cardiometabolic risk							
Body mass index (kg/m²)	22.2±3.8	23.3±3.8	23.4±3.8	23.2±3.9	23.1±3.6	23.2±3.8	0.261
TC (mg/dL)	201.0±33.4	200.8±33.3	200.3±33.9	201±33	200.4±33.5	202.7±33.6	0.188
LDL-C (mg/dL)	117.9±29.8	117.0±29.6	116.6±2.99	117.1±29.7	116.1±29.7	117.8±29.8	0.460
HDL-C (mg/dL)	62.5±15.9	62.4±15.7	62.1±15.9	62.0±15.6	62.7±16.0	63.0±13.4	0.273
TG (mg/dL)	97.1±58.7	96.4±58.0	97.8±60.2	97.1±58.1	96.4±58.5	97.7±60.0	0.908
Non-HDL-C (mg/dL)	138.5±33.2	138.3±32.9	138.3±33.5	138.9±33.1	137.6±33.3	139.7±33.2	0.422
sBP (mmHg)	118.1±15.0	117.7±14.5	118.2±14.4	117.8±14.4	118.4±14.3	118.4±14.9	0.602
dBP(mmHg)	75.1±11.6	74.9±11.8	75.0±11.7	75.0±11.5	75.1±11.5	75.4±11.6	0.734
Pulse rate (bpm)	72.0±10.8	71.9±10.9	71.9±11.0	72.0±10.7	71.9±10.8	72.1±10.6	0.982
HbA1c (%)	5.74±0.55	5.74±0.53	5.72±0.51	5.74±0.54	5.75±0.59	5.74±0.59	0.746
FBG (mg/dL)	98.9±16.3	98.9±16.2	98.6±15.1	98.7±15.7	99.0±17.2	99.1±17.3	0.902
Uric acid (mg/dL)	5.63±1.39	5.59±1.39	5.64±1.39	5.63±1.35	5.63±1.39	5.68±1.42	0.638
CRP (mg/dL)	0.03 (0.02/0.08)	0.02 (0.01/0.04)	0.03 (0.02/0.06)	0.03 (0.02/0.07)^{b}	0.04 (0.02/0.10)$^{b, c}$	0.07 (0.03/0.17)$^{b, e, g, h}$	<0.0001
Lifestyle behaviors							
Frequency of fish intake (days/week)	2.14±1.28	2.36±1.35	2.19±1.28^{a}	2.10±1.23^{b}	2.05±1.22$^{b, c}$	1.97±1.26$^{b, e, f}$	<0.0001
Amount of fish intake (g/week)	134±85	148±91	137±86^{a}	132±82^{b}	129±82$^{b, c}$	124±83$^{b, e}$	<0.0001
Cigarette smoking habit, n (%)	1,313 (14.6)	133 (7.3)	195 (10.7)	251 (14.4)	342 (18.2)	392 (22.7)	<0.0001
Aerobic exercise habit, n (%)	2,090 (23.3)	501 (27.7)	458 (25.2)	416 (23.9)	393 (20.9)	322 (18.6)	<0.0001

eGFR: estimated glomerular filtration rate, TC: total cholesterol, LDL: low-density lipoprotein, HDL: high-density lipoprotein, TG: triglyceride, sBP: systolic blood pressure, dBP: diastolic blood pressure, Hb: hemoglobin, FBG: fasting blood glucose, CRP: C-reactive protein
The amount of fish intake was defined as the average amount of fish intake per week. The frequency of fish intake was defined as an average number of days of fish intake per week. Aerobic exercise habit was defined as performing aerobic exercise more than 30 minutes at least twice per week.
ANOVA and post hoc tests with Tukey-Kramer correction and Games-Howell correction were performed to test between-group differences.
^{a}p<0.05, ^{b}p<0.0001 vs. G1
^{c}p<0.05, ^{d}p<0.001, ^{e}p<0.0001 vs. G2
^{f}p<0.05, ^{g}p<0.0001 vs. G3
^{h}p<0.0001 vs. G4

to confirm the interaction between the amount and frequency of fish intake, and lifestyle behaviors (aerobic exercise habit and cigarette smoking habit) on the WBC counts. A regression analysis was performed using the linear regression model and the estimate of Spearman's correlation coefficients.

We conducted all statistical analyses in this study using the SPSS software program, version 24 for Windows (SPSS Chicago, USA).

Results

Subjects

Population characteristics and the laboratory profiles according to WBC count as a categorical variable are shown in Table 1. Fig. 2 shows the average weekly amount of fish intake and average number of days per week of fish intake.

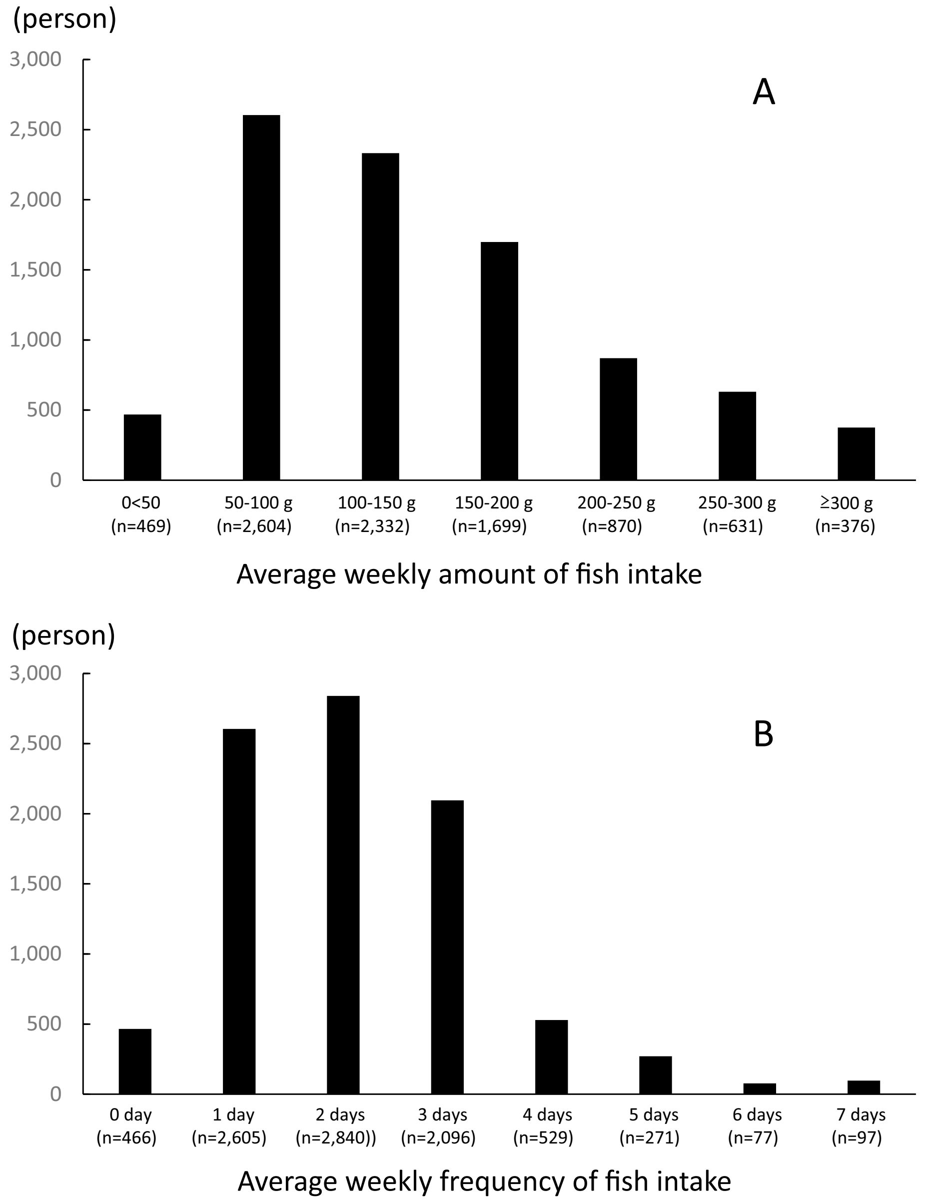

Figure 2. Frequency distribution of fish intake. A: weekly average amount of fish intake. B: weekly average numbers of days of fish intake. The average amount and frequency of fish intake were 134±85 g/week and 2.14±1.28 days/week, respectively.

Comparison of the subject characteristics and laboratory profiles according to WBC count

The serum C-reactive protein (CRP) level increased significantly as the WBC count increased ($p<0.0001$). The frequency of fish intake decreased significantly as the WBC count increased ($p<0.0001$). Similarly, the amount of fish intake decreased significantly as WBC count increased ($p<0.0001$). As the WBC count increased, the proportion of subjects who smoked increased significantly ($p<0.0001$), while those who regularly engaged with aerobic exercise decreased significantly ($p<0.0001$). No significant association was observed between the WBC count and other variables (Table 1).

The WBC count, regular aerobic exercise, and the amount and frequency of fish intake

As shown in Fig. 3-1, the WBC count was compared with the amount and frequency of fish intake. The WBC count decreased significantly as the amount of fish intake increased ($p<0.0001$ for trend) (Fig. 3A). Similarly, the WBC count tended to decrease as the frequency of fish intake increased ($p<0.0001$ for trend). (Fig. 3B). Moreover, as the amount and frequency of fish intake increased, the proportion of subjects engaged in regular aerobic exercise increased significantly (both $p<0.0001$ for trend) (Fig. 4A, B). However, the serum CRP levels, which were similar to the WBC counts over time, were not significantly related to the amount of fish intake or frequency of fish intake (data not shown).

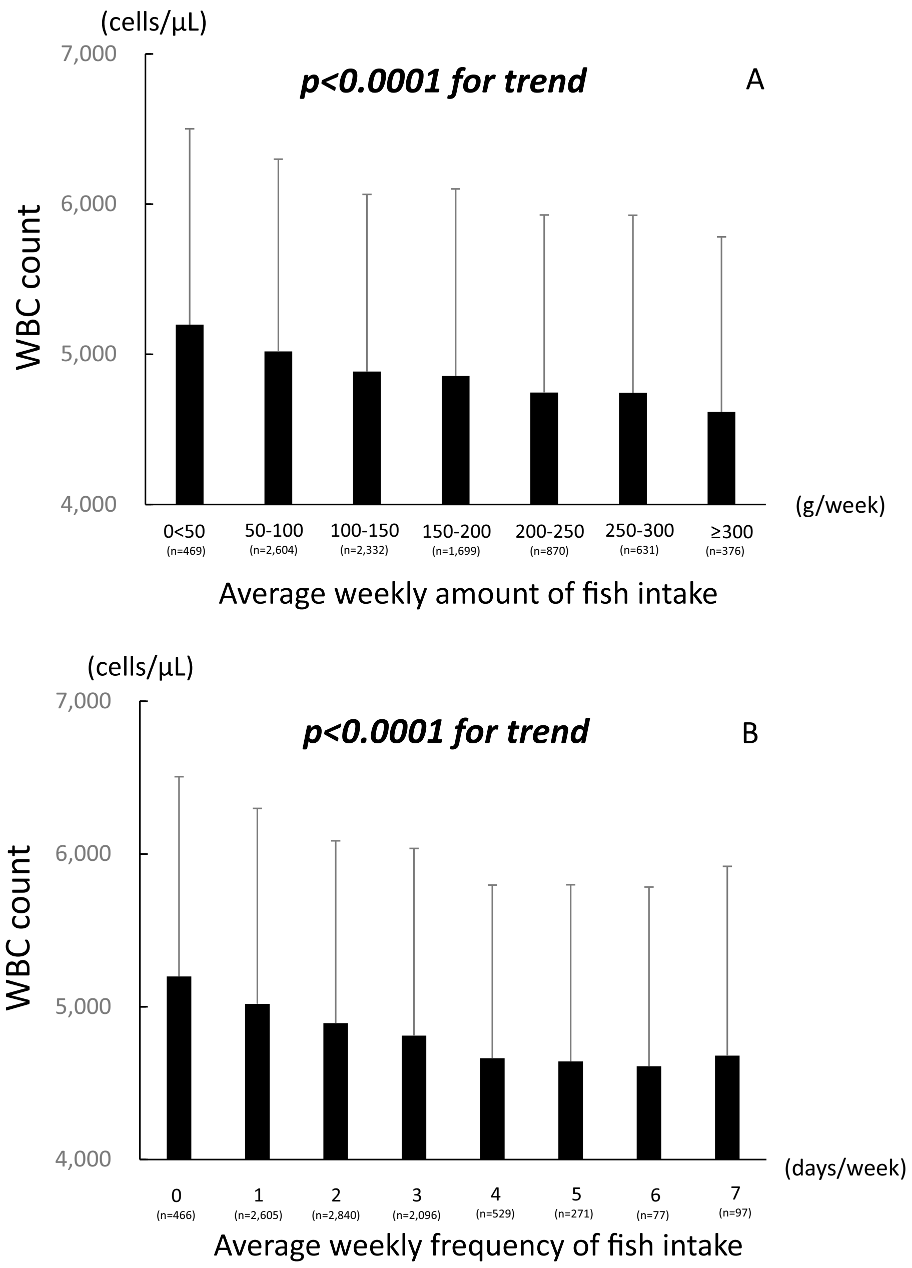

Figure 3. Association between the WBC count and fish intake. WBC: white blood cell. A: weekly average amount of fish intake. B: weekly average numbers of days of fish intake

Factors influencing the WBC count

As shown in Table 2, the amount of fish intake was negatively correlated with the WBC count (r=-0.092, p<0.0001). Lifestyle indicators, such as cigarette smoking (r=0.154, p<0.0001) and aerobic exercise (r=-0.077, p<0.0001), were significantly associated with an increased WBC count. These factors were included in the multivariate linear regression analysis model, which identified an increased amount of fish intake as an independent determinant of a low WBC count (β=-0.082, p<0.0001). Additionally, the aerobic exercise habit was an independent determinant of a low WBC count (β=-0.083, p<0.0001). Furthermore, cigarette smoking was an independent determinant of an increased WBC count (β=0.147, p<0.0001) (model 1).

A similar analysis was performed with the frequency of fish intake as the independent variable. The analysis showed that the frequency of fish intake was an independent determinant of low WBC count (β=-0.087, p<0.0001) (model 2). Thus, the amount and frequency of fish intake were significantly independent negative determinants of the WBC count, even after adjusting for cigarette smoking habit. These analyses indicated that fish intake and lifestyle behavior were independent determinants of WBC count. The relationship between the frequency and amount of fish intake and the proportion of subjects with cigarette smoking habits are shown in Fig. 3-3.

Furthermore, as shown in Fig. 4, 5, there was a significant correlation between the two indices of fish intake and lifestyle behaviors (aerobic exercise habit and cigarette

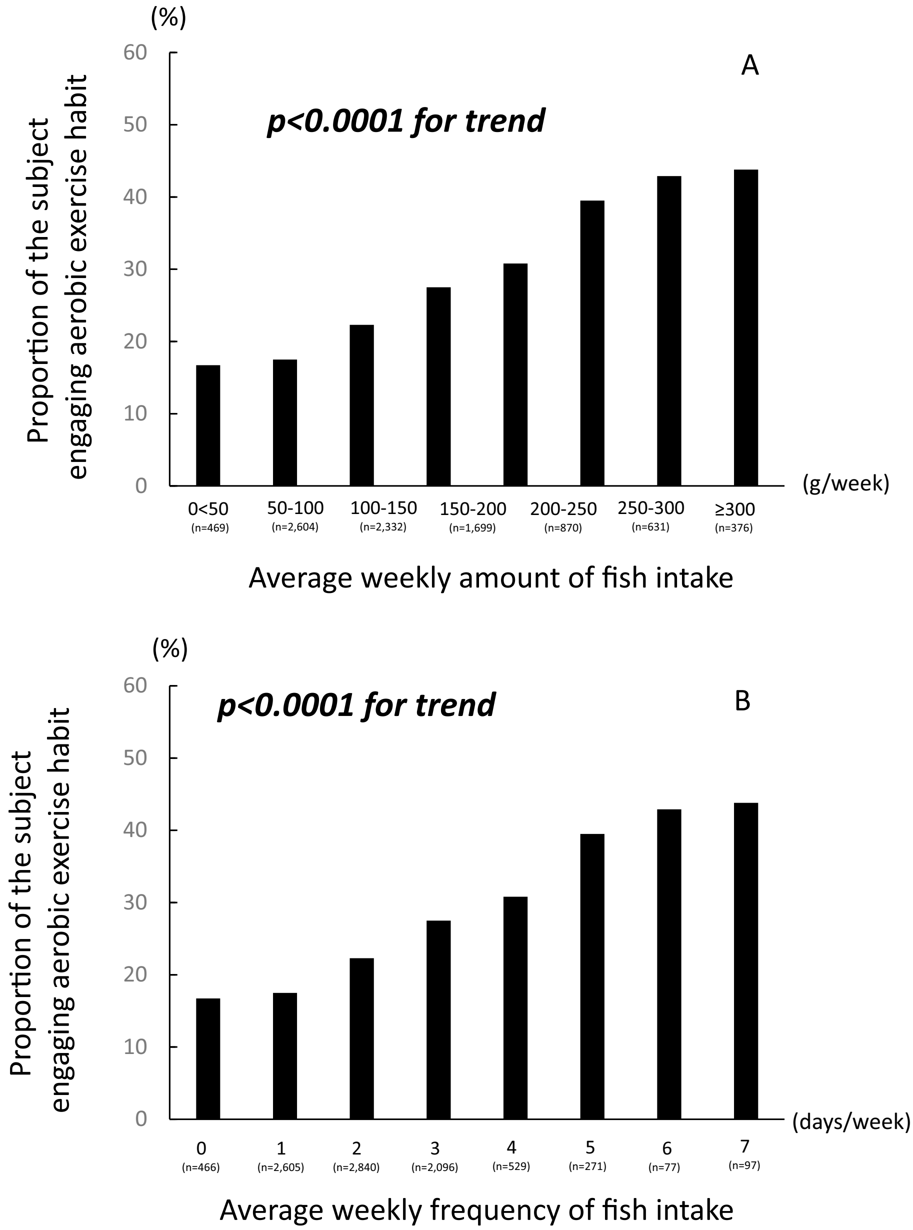

Figure 4. Association between aerobic exercise habit and fish intake. A: weekly average amount of fish intake. B: weekly average numbers of days of fish intake. Aerobic exercise habit was defined as performing aerobic exercise >30 minutes at least twice per week.

smoking habit); therefore, they could confound the association between the two indices of fish intake and WBC count. However, a two-way ANOVA revealed the absence of an interaction between the following two indices of fish intake and aforementioned lifestyle behaviors with respect to the relationship with the WBC count: (1) the amount of fish intake vs. aerobic exercise habit, amount of fish intake vs. cigarette smoking habit, aerobic exercise habit vs. cigarette smoking habit, amount of fish intake vs. aerobic exercise habit vs. cigarette smoking habit (p=0.399, 0.808, 0.229 and 0.307, respectively) and (2) the frequency of fish intake vs. aerobic exercise habit, the frequency of fish intake vs. cigarette smoking habit, aerobic exercise habit vs. cigarette smoking habit, the frequency of fish intake vs. aerobic exercise habit vs. cigarette smoking habit (p=0.505, 0.254, 0.596 and 0.997, respectively). Thus, these analyses indicate that lifestyle behaviors, and the two indices of fish intake, were independent determinants of the WBC count.

Fish intake and n-3 PUFA intake

We examined the correlation between the amount and frequency of fish intake and the n-3 PUFA intake. As shown in Fig. 6, the weekly average amount of fish intake and the weekly average number of fish intake measured in days were significantly correlated with the amount of estimated n-3 PUFA intake.

Discussion

This cross-sectional study found that the average fre-

Table 2. Univariate and Multivariate Linear Regression Analysis to Identify the WBC Count.

Dependent variable: WBC count			**Model 1** (n=8,981)		Dependent variable: WBC count			**Model 2** (n=8,981)	
	Univariate		Multivariate			Univariate		Multivariate	
	r	p value	β	p value		r	p value	β	p value
Age	0.015	0.148			Age	0.015	0.148		
Male gender	0.018	0.096			Male gender	0.018	0.096		
eGFR	0.010	0.374			eGFR	0.010	0.374		
Body mass index	-0.015	0.160			Body mass index	-0.015	0.160		
TC	0.013	0.209			TC	0.013	0.209		
LDL-C	0.003	0.750			LDL-C	0.003	0.750		
HDL-C	0.018	0.085			HDL-C	0.018	0.085		
TG	-0.001	0.900			TG	-0.001	0.900		
Non-HDL-C	0.008	0.470			Non-HDL-C	0.008	0.470		
sBP	0.010	0.357			sBP	0.010	0.357		
dBP	0.011	0.295			dBP	0.011	0.295		
Pulse rate	0.004	0.725			Pulse rate	0.004	0.725		
HbA1c	0.003	0.776			HbA1c	0.003	0.776		
FBG	0.005	0.646			FBG	0.005	0.646		
Uric acid	0.015	0.195			Uric acid	0.015	0.195		
Lifestyle behaviors					***Lifestyle behaviors***				
Amount of fish intake	-0.092	<0.0001	-0.082	<0.0001	**Frequency of fish intake**	-0.092	<0.0001	-0.087	<0.0001
Cigarette smoking habit	0.154	<0.0001	0.147	<0.0001	Cigarette smoking habit	0.154	<0.0001	-0.146	<0.0001
Aerobic exercise habit	-0.077	<0.0001	-0.083	<0.0001	Aerobic exercise habit	-0.077	<0.0001	-0.061	<0.0001
Multiple R=0.187, F=108.030, p<0.0001					Multiple R=0.189, F=110.452, p<0.0001				

Abbreviations are as in Table 1. r: correlation coefficient, β: standard partial regression coefficient

The amount of fish intake was defined as an average amount of fish intake per week.

The frequency of fish intake was defined as an average number of days of fish intake per week.

Aerobic exercise habit was defined as performing aerobic exercise more than 30 minutes at least twice per week.

quency of fish intake, measured in days per week, and average amount of fish intake per week were independent determinants of the WBC count. Moreover, aerobic exercise habit, which increased with an increase in the amount and frequency of fish intake, was an independent determinant of low WBC count. Moreover, we obtained similar results using both measures of fish intake (amount and frequency). Since the frequency of fish intake can be more easily obtained from a questionnaire than the amount of fish intake; therefore, it can be used as a general measure of fish intake.

As shown in Fig. 6, the positive association between the amount and frequency of fish intake and the amount of n-3 PUFA intake, which had cardioprotective and anti-inflammatory effects (5, 17-20), suggested that the two measures of fish intake had a similar amount of n-3 PUFA intake. It may be presumed that this phenomenon accounted for the similar anti-inflammatory effects of both a high amount and a high frequency of fish intake. In other words, the amount and frequency of fish intake may be equivalent indicators of anti-inflammatory activity. We already reported that the elevation of the eicosapentaenoic acid/arachidonic acid ratio, which can be derived from the daily fish intake, was an independent predictor of a reduced WBC count, indicating low-grade chronic inflammation (21). This report may support our results.

We obtained similar results using the two measures of fish intake in a relatively large cohort, which increased the validity of our results. Therefore, the amount and frequency of fish intake had a positive correlation. Several large cohort studies that investigated the protective effects of fish intake on CAD calculated the amount of fish intake based on the average number of fish intake, measured in days per week (3, 4). As shown in Fig. 2, in a study with a large sample size, the frequency distribution of each category of fish intake was nearly equal. Thus, the amount and frequency of fish intake can be measures of fish intake with equivalent results. There may be no difference in the outcome, even in a study with the same subjects.

Several studies have reported that a dietary habit involving a high fish intake was positively related to a healthy lifestyle, such as regular aerobic exercise and no cigarette smoking habit (22-24). The present study showed that the three measures of fish intake, aerobic exercise habit, and cigarette smoking habit were independent determinants of WBC count. The suppressive effect of fish intake on ASCVD development was attributed to the additive or synergistic effects of high fish intake and a healthy lifestyle on atherosclerosis progression (25, 26). Furthermore, we confirmed that fish intake, an aerobic exercise habit, and a cigarette smoking habit do not interact with each other in relation to the WBC count. We showed that each factor is independently associated with the WBC count. If an allocation test with fish intake as an intervention was conducted, it was unlikely that the fish intake group's lifestyle would have

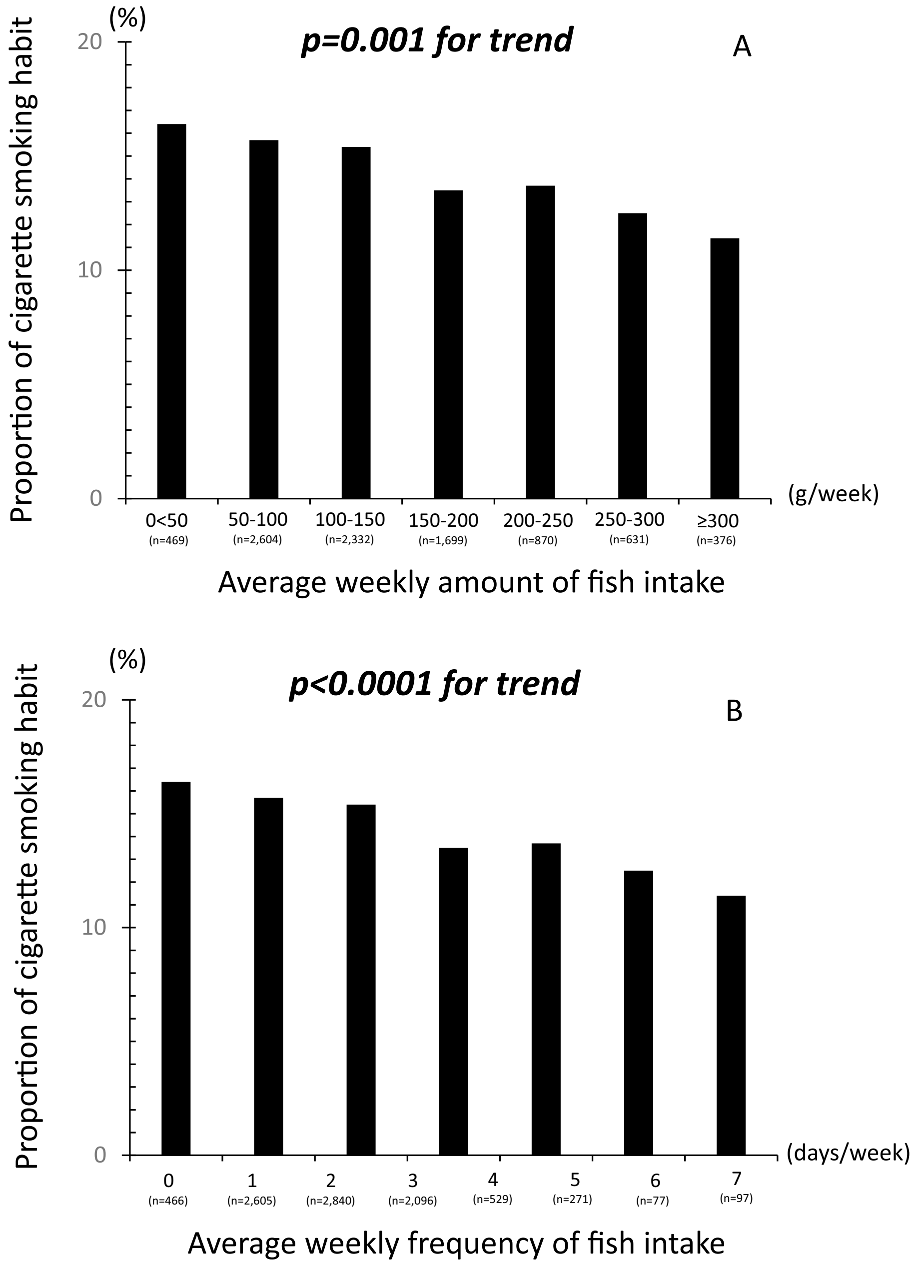

Figure 5. Association between cigarette smoking habit and fish intake. A: weekly average amount of fish intake. B: weekly average numbers of days of fish intake.

changed.

The results of analyses in this study showed no significant difference in any association or outcome regardless of the measure of fish intake that the investigators used. However, if the study focused on eating habits with high fish intake and other lifestyle habits, it may be better to use the average number of fish intake measured in days per week rather than measuring the average amount of fish intake per week. Moreover, the frequency of fish intake can be more easily obtained than the amount of fish intake. Therefore, to measure the frequency of fish intake can be more useful in relatively large-scale studies. The frequency of weekly fish intake necessary to prevent cardiovascular events was recommended in a scientific statement by the American Heart Association to be once to twice a week (6). However, it is necessary to evaluate various outcomes to examine the usefulness of this measure of fish intake. However, the two indices of fish intake may both be generally useful. The decision of which index to use for fish intake should consider other factors, such as research facilities, study design, and the situation.

This study is associated with several limitations. First, since this was a cross-sectional study, there were no causal associations established. Second, the amount of fish and n-3 PUFA intake in this study was recalculated by the National Institute of Health and Nutrition based on data obtained from a national nutrition survey conducted under the initiative of the NHLW on a randomly selected population. This provided reliable data, but it may not have accurately reflected the actual fish intake and n-3 PUFA intake of the

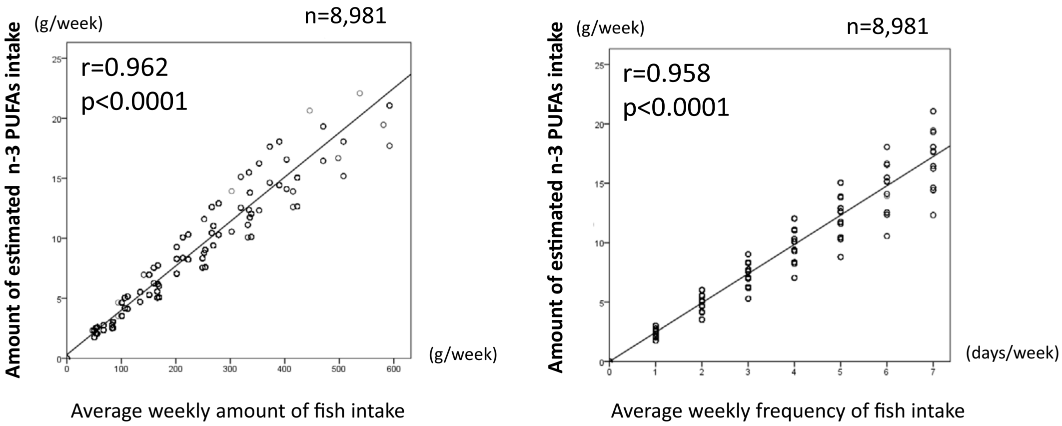

Figure 6. Relationship between fish intake and amount of n-3 PUFAs intake. PUFA: polyunsaturated fatty acid

subjects. Third, the present study failed to find an association between the serum CRP levels, a predictive marker of ASCVD, and fish intake. We aim to elucidate this mechanism in a future study. Finally, it may have been beneficial to prepare a detailed internationally standardized questionnaire (27, 28) or used standard variables to facilitate international comparisons (lifestyle, dietary habits, and physical activity).

Conclusion

A high fish intake is an independent determinant of low WBC counts and aerobic exercise habits (regardless of whether it is defined as the average weekly amount of fish intake or the weekly average number of days of fish intake). These results suggest that frequency can be obtained more easily than the amount of fish intake; hence, it can be used as a substitute for the amount of fish intake. However, we propose that it should be selected according to the research design, facilities, and situation to measure fish intake.

The authors state that they have no Conflict of Interest (COI).

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